Project Implementation[1]

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[1] from https://2022.igem.wiki/sheffield/implementation

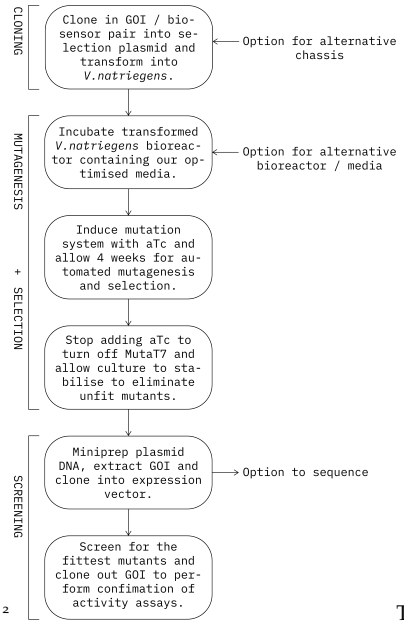
Explain how you would implement your project in the real world

rEvolver Workflow

The plasmids will be designed with appropriate restriction sites in such a way to allow the user to clone in their GOI into the flanking T7 promoter region in addition to an appropriate biosensor element upstream of the growth modulation system. Initially we considered scarless cloning in the final rEvolver design, but ultimately came to the conclusion that restriction sites should remain to allow users to swap genetic parts as their project adapts. We will give users the option of using a negative selection system (fitness relates to down-regulation of growth slowing genes) or positive selection system (fitness relates to upregulation of alternative carbon source metabolism). Although protein evolution by the rEvolver is limited by the availability of an appropriate biosensor, it is important to be aware of the huge array of biosensors that already exist, and how the rate of their discovery continues to accelerate.

Once users have cloned a GOI and relevant biosensor into the selection plasmid, they will transform all three plasmids into a desired chassis – this could be standard laboratory E. coli, but we have optimised and characterised many of the genetic parts for compatibility in Vibrio natriegens to allow for faster evolutionary progress. If using V. natriegens, this may involve using a mathematically modelled and designed media that optimises laboratory strains of V. natriegens for fast growth – the recipe of which would be provided. Since the directed evolution would occur completely in vivo, the next step is to simply grow the transformants in our easy to build bioreactor. Following evolution, the end users would be required to screen for the fittest mutants, and isolate the GOI to confirm enzymatic activity. These could vary hugely depending on the GOI, but are important steps to verify protein optimisation.[2]

We have constructed our testing plasmids through HiFi assembly which is something we want to omit from the final designs to allow for a more modular system. Traditional restriction enzyme cloning that leaves the restriction site following cloning would allow users to swap in and out genetic parts as needed at a greatly reduced cost in comparison to a hifi assembly. Upon successfully achieving this, we would need to publish our plasmids on a repository like AddGene. It is also possible there may be some issues having three plasmids in



[2] Typical workflow we would expect the end user to follow using rEvolver. Arrows show different options for using different strains or bioreactors.

the same cell simultaneously as triple transformations are notoriously difficult to accomplish. Additionally, the fact that the gene of interest exists on a plasmid may mean there are several copies of it in a cell even if the plasmids are low copy number. Whilst we know all of these parts work in isolation as they have been shown to do in the literature, we have not yet tested the full proposed system with both mutagenesis and selection in the same sample. Finally, although we were successful in predicting V. natriegens growth rate in our Design of Experiments phase, we would prefer to perform a few more iterations to hone in on the perfect media before we release this to the public.

Open Source Bioreactor Assembly

When we first chose ingredients to include in our test media recipes, we focused on individual ingredients that looked beneficial for growing *V. natriegens*, but we did not consider how they would react. The purpose of this design was to efficiently screen through potentially beneficial *V. natriegens* growth media ingredients, using a statistical model whose output would be a linear model with the estimated effect sizes and p-values (significance) of the ingredients on growth rate. At this stage, we had not seen statistical design of experiments used to systematically find an optimal growth media for V. natriegens in the literature. We have seen similar methods used to optimise media for other microorganisms.

We have made an easy to follow assembly guide, if the user wishes to use our toroidal bioreactor. Our bioreactor is aimed at users of all skill levels. The affordable materials and components, and the open source code all lend themselves to opening up synbio to a wider audience. Being a table-top turbidostat allows for its use in all kinds of settings, from laboratories to classrooms to home garages. The MicroPython software is intuitive and versatile, theoretically allowing future users to adapt our turbidostat for a wide range of purposes beyond directed evolution/maximal growth maintenance (including just using it as a regular chemostat).

Theoretically users could scale our design up beyond 100mL culturing instances. Increasing the volume by lengthening the circle into an oval would preserve the cross-sectional area and in turn maintain the same heat loss and oxygenation rates. However, this could be challenging to construct. The existing construction guide would have to be altered to use sheets of perspex the same thickness as the cylinders, and with the cylinders being cut down the middle and joined to the flat sheets. The lid and base would follow the basic construction methods, again just with lengthening them to fit over the main vessel

properly.

However, significant scaling up might be beyond some of the components we have opted for. Raspberry Pi Pico and MicroPython are reliable structures for relatively simple functionality and scale, but may struggle with larger scales. The peristaltic pump would likely have to be upgraded (or multiple pumps could be used simultaneously). The magnetic stirring system would have to be replaced with something akin to a bike chain or a rail underneath to still guide the stirring magnets around properly, as the shape of the bioreactor would no longer be a circle and hence not fit for a simple servo and horn arrangement.

Construction of our bioreactor comes with the regular risks inherent with using hand and power tools, electronics and circuitry. Risks of abrasions, burns, cuts and zaps are always present, and this is alongside the regular risks associated with culturing prokaryotes. Regular caution, a respect for the tools one is using and a desire for self-preservation is always advisable. There is also always the small risk of malware when relying upon online resources, so users should always keep their wits about them when sourcing code online.

Open-Source Bioinformatics Toolbox

The bioinformatics toolbox has been designed to find a good balance between user friendliness and versatility – primarily to unify many of the staple tools that geneticists and synthetic biologists routinely use and streamline them into a common, navigable interface.

Currently our web-based toolbox is capable of performing the following functions upon a given DNA, RNA, or amino acid sequence:

- Determining length, GC content and sequence type of all inputs
- Converting between upper and lower case
- Reversing the sequence
- Counting element frequencies (bases or residues)
- Reverse complementing DNA and RNA
- Converting between DNA, RNA and amino acid sequences
- Finding Open Reading Frames (ORFs)

Additional tools that have been developed and incorporated in the underlying Rust library are:

- Subsequence isolation

- Calculating Hamming and Levenshtein distances

We feel that this is an impressive array of functionality for a toolbox that also features a clean, intuitive UI. It is also worth mentioning we designed our toolbox to be mobile friendly – so people can perform quick calculations on the go. We know that future Sheffield iGEM iterations will definitely use it going forward, and we feel confident that our toolbox will become a favourite for many others outside of our university!

Finally, since our toolbox is developed on GitHub in a completely open-source manner, any future users of our toolbox could contribute tools and improvements of their own. In this way, we can guarantee that our software tool will continue to grow and evolve independent of our input — a contribution that genuinely belongs to the community.

References

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